complexes hierarchically, at different times, with variable stoichiometry within the adhesion itself and change as the adhesion matures into larger structures. To parse out the effects of the mechanical properties of the ECM on the numbers, aggregation states, and associations of these molecules, we extend these measurements to substrates with variable stiffness and correlate them with high-resolution traction force microscopy (TFM) measurements. We show that individual and newly formed adhesions at the leading edge of protruding cells transmit forces on soft as well as stiff substrates, with force magnitudes that correlate with the integrated intensity of the adhesions and the total number of individual adhesion molecules. These measurements provide novel information on complex formation as adhesions evolve and respond to substrate rigidity.

2493-Plat
Tracking Rotation during Leukocyte Rolling Reveals Asymmetric Adhesion Properties
Isaac T.S. Li[1,2], Taekjip Ha[1,3], Yann R. Chemla[1,2].
[1]Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Center for the Physics of Living Cells, Urbana, IL, USA, [3]Howard Hughes Medical Institute, Urbana, IL, USA.
Leukocytes are responsible for fighting infections in the body. When injuries occur, selectin molecules are expressed on the surface of nearby blood vessel walls. These selectin molecules transiently adhere to leukocytes flowing in the bloodstream and capture them, leading to leukocyte rolling towards the injury site. Rolling adhesion is critical for leukocytes to locate injury sites and to activate various signaling pathways for subsequent transmigration and chemotaxis.
Individual adhesion components involved in rolling adhesion, such as adhesion molecules and membrane tethers have been characterized. However, models incorporating these properties are still unable to describe fully the rolling behavior. Current models assume uniformly distributed adhesion properties on cell surfaces due to the lack of measurements quantifying this distribution. Here, we determined experimentally the spatial distribution of adhesive properties on leukocyte surfaces. We used dark-field imaging and particle tracking techniques to extract not only the translational but also the rotational motion of a single rolling cell. The additional rotational information allows us to map precisely the whole cell motion and adhesion properties to a particular cell orientation. We find that the adhesion properties of the leukocyte surface are far from homogenous, with large, localized patches on the cell surface exhibiting strong or weak adhesive properties. This finding provides new insight into leukocyte adhesion properties, such as the asymmetric distribution of receptor and microvilli on rolling cells, and could lead to better modeling of rolling adhesion.

2494-Plat
Chromatin Association with the Nuclear Envelope Supports Stable Nuclear Mechanics
Sarah M. Schreiner[1], Peter K. Koo[2], Yao Zhao[3], Simon G.J. Mochrie[2], Megan C. King[1].
[1]Cell Biology, Yale University, New Haven, CT, USA, [2]Physics, Yale University, New Haven, CT, USA, [3]Applied Physics, Yale University, New Haven, CT, USA.
Although the nuclear lamina is considered to be the primary mechanical defense of the nucleus, lamins are part of an integrated network of lipids, proteins, and chromatin. Here, we isolate the contribution of chromatin to nuclear mechanics by employing fission yeast, which lack a nuclear lamina. We have combined a quantitative imaging platform capable of measuring 3D nuclear contours *in vivo* with an *in vitro* optical tweezers assay to probe the mechanical properties of *S. pombe* nuclei. In live cells, we find that association of chromatin with the inner nuclear membrane (INM) through integral membrane proteins is required for a normal mechanical response to microtubule (MT) forces. Increasing loss of integral INM proteins results in highly deformable nuclei specifically in response to exogenous MT forces. These nuclei also show a decreased capacity to recover from mechanical stress. Using optical tweezers, we find that nuclei lacking integral INM proteins are less stiff than wild type nuclei and have increased chromatin flow, particularly when force is applied at rates that recapitulate the kinetics of MT dynamics *in vivo*. Wild type mitotic nuclei, in which chromatin is globally released from the INM, are extremely soft and also display increased chromatin flow compared to interphase nuclei. Interestingly, decreasing the chromatin to nuclear volume ratio without altering the association of chromatin with the INM has only a slight effect on stiffness and does not alter chromatin flow. Together, these findings suggest that association of chromatin with the nuclear envelope underlies nuclear stiffness. Further, release of chromatin from the INM allows chromatin to flow into MT-dependent fluctuations of the nuclear envelope, leading to larger, longer-lasting nuclear deformations. Lastly, these results suggest that chromatin association with the lamina may contribute to the mechanical behavior of metazoan nuclei.

2495-Plat
Three-Dimensional Balance of Cortical Tension and Axial Contractility Enables Fast Amoeboid Migration
Begona Alvarez-Gonzalez[1], Ruedi Meili[2], Effie Bastounis[2], Richard A. Firtel[2], Juan C. Lasheras[1], Juan C. del Alamo[1].
[1]MAE, UCSD, La Jolla, CA, USA, [2]Cell and Developmental Biology, UCSD, La Jolla, CA, USA.
Fast amoeboid migration requires cells to apply mechanical forces on their surroundings via transient adhesions. However, the mechanistic role of forces in controlling cell shape changes and cell migration speed remains largely unknown. To address these questions, we used three-dimensional force microscopy to measure the in-plane (tangential) and out-of-plane (normal) forces exerted by cells crawling on flat surfaces. From the measured normal forces, we estimated the cells' cortical tension using a Young-Laplace's model. We examined chemotaxing wild-type *Dictyostelium* cells, as well as mutants with defects in contractility, internal F-actin crosslinking, and cortical integrity, and demonstrated that once the cells initiate their migration and polarize, they generate tangential traction forces by myosin II contractility, which requires an internal crosslinked F-actin network. Simultaneously, cortical tension provides an additional mechanism that generates the normal forces and that does not require myosin II. The 3-D pulling forces generated by both mechanisms are internally balanced by an increase in cytoplasmic pressure that allows cells to push down on their substrate without adhering to it. These compressive pressure-induced forces are not associated to adhesion sites, and may allow amoeboid cells to push off surrounding structures when migrating in complex three-dimensional environments. Our findings are consistent with a model in which the two force-generating cellular domains are mechanically connected by myosin I crosslinking that enables the communication of forces between the domains. Furthermore, we found that the balance between axial myosin II contractility and cortical tension is important to produce the cell shape changes needed for locomotion, as cell migration speed correlates with the ratio of the magnitudes of the tangential traction forces to the normal ones. These results reveal a novel role for 3-D cellular forces in establishing the efficiency of amoeboid cell movement.

2496-Plat
Measuring Mechanical Force during Zebrafish Development using an Expressible Tension Sensor
Andrea Hamilton[1], Victoria Wu[2], Alex Dunn[3], Ingmar Riedel-Kruse[2].
[1]Molecular and Cellular Physiology, Stanford University, Palo Alto, CA, USA, [2]Department of Bioengineering, Stanford University, Palo Alto, CA, USA, [3]Department of Chemical Engineering, Stanford University, Palo Alto, CA, USA.
Early vertebrate development is a mechanically dynamic process. Embryos undergo radical morphological changes to mold a ball of cells into the recognizable planes of a frog or fish or mouse. Along with more basic morphogenesis, evidence exists that mechanical force can play a determinative role in processes such as differentiation and tissue sorting. Yet, while the chemical and genetic signals underlying early development have long been studied, the mechanical stresses defining the physical landscape of the embryo remain poorly understood, particularly at the cell and molecule scale. An expressible fluorescence resonance energy transfer (FRET) based probe has been developed to measure piconewton force levels in vivo (Grashoff et al. 2010). We adapt this Tension Sensor Module (TSMod) for use in the zebrafish embryo using the epithelial cell adhesion molecule, EpCAM. In doing so we validate FLIM-FRET for use in zebrafish embryos and show that TSMod can be used to make meaningful cell and tissue scale force measurements in a developing vertebrate system.

2497-Plat
Two Distinct Regimes of Integrin Molecular Forces
Xuefeng Wang, Taekjip Ha.
physics, university of illinois at urbana-champaign, Urbana, IL, USA.
Forces transmitted by integrins activate focal adhesion kinase (FAK) and regulate cell adhesion, spreading and migration. However, the magnitudes and sources of the molecular forces involved with these mechanical regulations remain largely unknown. Here we discovered two distinct regimes of integrin molecular forces generated by cell membrane and actomyosin, respectively, and that they govern different cellular functions. Using integrin ligands linked with tension gauge tether (TGT), we showed that TGT with 54 pN tension tolerance (T_{tol}) is specifically ruptured at focal adhesions (FA) in CHO-K1 cells or central regions of cell-substratum contact area in neutrophil-like dHL-60 cells, indicating that remarkably strong forces can be applied to a single integrin. This